Periphyton succession in a waste water treatment pond

Sucesión del perifiton en un tratamiento de aguas residuales

Alex Chuks CHINDAH [1], Solomon Amabaraye BRAIDE[1], Jonathan AMAKIRI[2] and Oluwakemi Okoba KIOLAWSON AJIBULU[1]

[1]Institute of Pollution Studies, Rivers State University of Science and Technology, Nkpolu Oroworukwo P M B 5080, Port Harcourt, Rivers State , Nigeria and [2]Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State, Nigeria. E-mails: alexchindah@yahoo.com Corresponding author

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ABSTRACT

A study on periphyton succession in the self-depuration wastewater body exposed to sunlight was conducted for 15 days in a laboratory pond. The physico-chemical parameters (temperature, pH, salinity, conductivity, turbidity, total dissolved solids, total suspended solids, nitrate, phosphate and sulphate) and biological parameters (periphyton) were determined. The changes observed in some of the physico-chemical variables indicated a reduction in biochemical oxygen demand (BOD) (96%), chemical oxygen demand (COD) (96%), nitrate (93%), phosphate (81%) and sulphate 55%. Periphyton standing stock was 6.31 x 10^6 indiv L^{-1} at day 15. pH and dissolved oxygen (DO) showed strong linearity with periphyton standing stock and biomass. The standing stock and biomass had a positive relationship with the species dominance index, and an inverse relationship with species diversity. Linear regression model predicted 70% and 64% potential changes in the periphyton biomass that might be attributed to pH, DO and BOD, and NO_3^-, PO_4^{-3}, and SO_4^{2-}, respectively. The periphyton assemblages shifted in dominance from one algal form to another through out the exposure time, with a total of 50 algal species encountered during the study. The successional patterns of the periphyton community revealed that *Oscillatoria terebriformis, Lyngbya pseudospirulina, Chlamydomonas reinhardtii, Euglena pascheri, Lepocinclis steinii* and *Oscillatoria chalybaea* are useful as bioindicators of municipal wastewater.

Key words: Periphyton, Succession, waste water, treatment, sunlight

RESUMEN

Se condujo un estudio sobre la suceción del perifiton en la autodepuración de un cuerpo de aguas residuales expuesto a la luz solar durante 15 días en un estanque tipo laboratorio. Se determinaron parámteros físicos (temperatura, pH, salinidad, conductividad, turbidez, sólidos disueltos totales, sólidos suspendidos totales, nitrato, fosfato y sulfato) y parámetros biológicos. Los cambios observados en algunas de las variables físico-químicas indicaron una reducción en la demanda bioquímica de oxígeno (DBO) (96%), demanda química de oxígeno (DQO) (96%), nitrato (93%), fosfato (81%) y sulfato (55%). El máximo standing stock de 6.31 x 10^6 indiv L^{-1} se observó para el perifiton Algunos parámetros físico-químicos tales como pH y oxígeno disuelto (OD) mostraron una fuerte asociación lineal con el standing stock del perifiton y la biomasa. El standing stock y la biomasa tuvieron una relación directa positiva con el índice de la dominancia de especies pero exhibieron una relación inversa con la diversidad de especies. Los patrones sucecionales de la comunidad del perifiton revelaron que that *Oscillatoria terebriformis, Lyngbya pseudospirulina, Chlamydomonas reinhardtii, Euglena pascheri, Lepocinclis steinii* y *Oscillatoria chalybaea* son útiles como bioindicadores de las aguas residuales municipales. El modelo de regresión lineal predijo los cambios potenciales en la biomasa del perifiton que puede ser atribuido al pH, DO y BOD en 70% y NO_3^-, PO_4^{-3} y SO_4^{2-} en 64%. La composición del perifiton cambió en la dominancia de una forma algal a otra a través del tiempo de exposición con un total de 50 especies del perifiton encontradas durante el estudio.

Palabras clave: Perifiton, succesión, aguas residuales, tratamiento, luz solar.

INTRODUCTION

The increasing human population and activities such as expansion of urban centres and industrial setups have resulted in the generation of different waste types that are discharged into surface water bodies. Much of these are in solid and liquid forms consisting of domestic organic and inorganic wastes, spent oil (crank caseoil), and agricultural pesticides and fertilizers. The magnitude of these wastes has in recent times increased several folds and is now of concern to all the stakeholders including the scientific community (SC), non-governmental agencies (NGO), government agencies (GA) and other citizens (Chindah 1998).

One of the freshwater bodies impacted by these activities is the Nta-wogba stream that receives several point and non-point sources of untreated industrial and municipal wastes. The stream finally empties into the brackish water bodies where its impacts on water quality and biological resources resulting in loss of water integrity, aesthetics and biodiversity.

This freshwater ecosystem is *apriori* capable of self-purification through biological processes (Lakatos *et al.,* 1997), which depends largely on the physiographic features of the stream and climatic conditions as wastes received and discharged are within the carrying capacity of the system (Soler *et al*., 1991). Under this circumstance, the effluent load is small and thus capable of elimination of organic and inorganic pollutants by decomposition and the absorption of inorganic compounds, through the simultaneous physicochemical and biological processes (EPA 1983, 1987). With the discharges from these municipal and industrial settings being overwhelming, results in the inability of the system to carry the extraneous organic and inorganic load with the concomitant loss of integrity and the goods and services which it provides.

In spite of increasing trend in the magnitude of wastes discharged into the natural environment and the threat posed to these resources as a result of human activities, little has been achieved in respect of waste treatment process and the physicochemical and biological interplay in Nigeria (Chindah *et al*., 2005; Chindah *et al.,* 2007). Most of the previous studies mainly focused on the status of water qualities (RPI, 1985, IPS 1990, NDES, 2000, NDDC, 2004,) and level of contaminants on water resources (Ajayi and Osibanjo, 1981; Ndiokwere 1984; Ibiebele *et al*., 1987; Ekweozor *et al.,* 1987; Powell 1987; Ekweozor *et al.,* 1987; Amadi *et al.*, 1997; Okpokwasili and Nwabuzor 1988; Okpokwasili and Olisa, 1991; Chindah, 1998; Joiris and Azokwu 1999; Chindah and Sibeudu, 2003).

Little is however known on wastewater self–depuration that practically requires no external energy other than sunlight, as well as, oxygen which is essential for the decomposition of organic matter and is provided in high proportion by the photosynthetic activities of the microbial communities present in the system (Abeliovich 1986).

Greater efficiency of the treatment is achieved when the microbes used in the treatment process are aerobic bioreactors (algae, protozoa or bacteria) and their optimum environmental conditions for growth provided (EPA 1990 and 2002).

Some of these studies have implicated periphyton as possible candidate in wastewater treatment and are gaining worldwide attention (Soler *et al*., 1991; Laktos *et al.,* 1997; EPA 2002). In most developed world, the use of stabilization ponds as a biological system has assumed great importance based on its economy in wastewater management and usefulness in production of microorganisms that mineralize the organic and inorganic components (Oswald 1988 and Ogan 1988).

In order to bridge the existing gap at Nta-Wogba is located on the western flank of Port Harcourt city of the Rivers State, Nigeria this area, this study was undertaken to monitor water quality and successional patterns of periphyton assemblages with the view of identifying possible indicator species relating to changes in water quality during the treatment process.

MATERIALS AND METHODS

Study area

The Nta-Wogba is located on the western flank of Port Harcourt city of the Rivers State, Nigeria. The stream lies between latitude 40 50" and 50 00"N and longitude 60 55" and 70 00"E (Figure 1.). The climate of the area is that of tropical equatorial latitude with rainfall occurring almost all year round (Gobo 1998; Gobo *et al.,* 2008). The Nta-wogba is a black water stream with its head water draining the Ora-Azi forest, and meanders through the densely populated city of Port Harcourt into the Bonny estuary.

The stream system is exposed to increasing amount of urban wastes as it flows seaward, mainly from industrial and domestic discharges from laundry, photographic studios, garages, and wastes from markets and construction sites. The human activities exert considerable negative impact on the entire study area. It is estimated that the water body receives about 4500 L/day of waste containing petroleum product, especially from crankcase oil, over 250,000 L/day from domestic waste, 80 kg/day of human waste, 20 kg/day of metal, and 58 kg/day of solid waste such as paper and polyethylene bags.

Rainfall occurs almost all the months (May - November) of the year with short duration of dry season (December -April) and an annual average rainfall of 2360mm (Gobo 1988 and Gobo *et. al*, 2008). The natural drainage basin is largely exposed as vegetation is virtually removed by adjacent development with the fringe and water surface covered by macrophytes such as *Nymphaea micrantha, N. lotus, Pistia stratiotes, Eclipta prostrate, Torulinium odoratum, Ludwigia leptocarpa, L. erecta*, *Ipomea aquatica, Neptuna oleracea , Saccioleis Africana, Cyperus distans,* and *C. sphacelatus* (Chindah *et al.*, 2005; Izonfuo *et al.,* 2005)

Experimentation

Water from the study station was collected in pre-cleaned 50 litre plastic jerry cans to fill two triplicate 50 L polyethylene tanks in the laboratory. The tanks were left in an open and wide area to avoid shading at all times. From the tanks, samples for water quality and biological analysis were conducted for a period of two months. Slide panels in rack were placed in each of the tanks. The slides were examined under binocular microscope on each day for the assessment of periphyton.

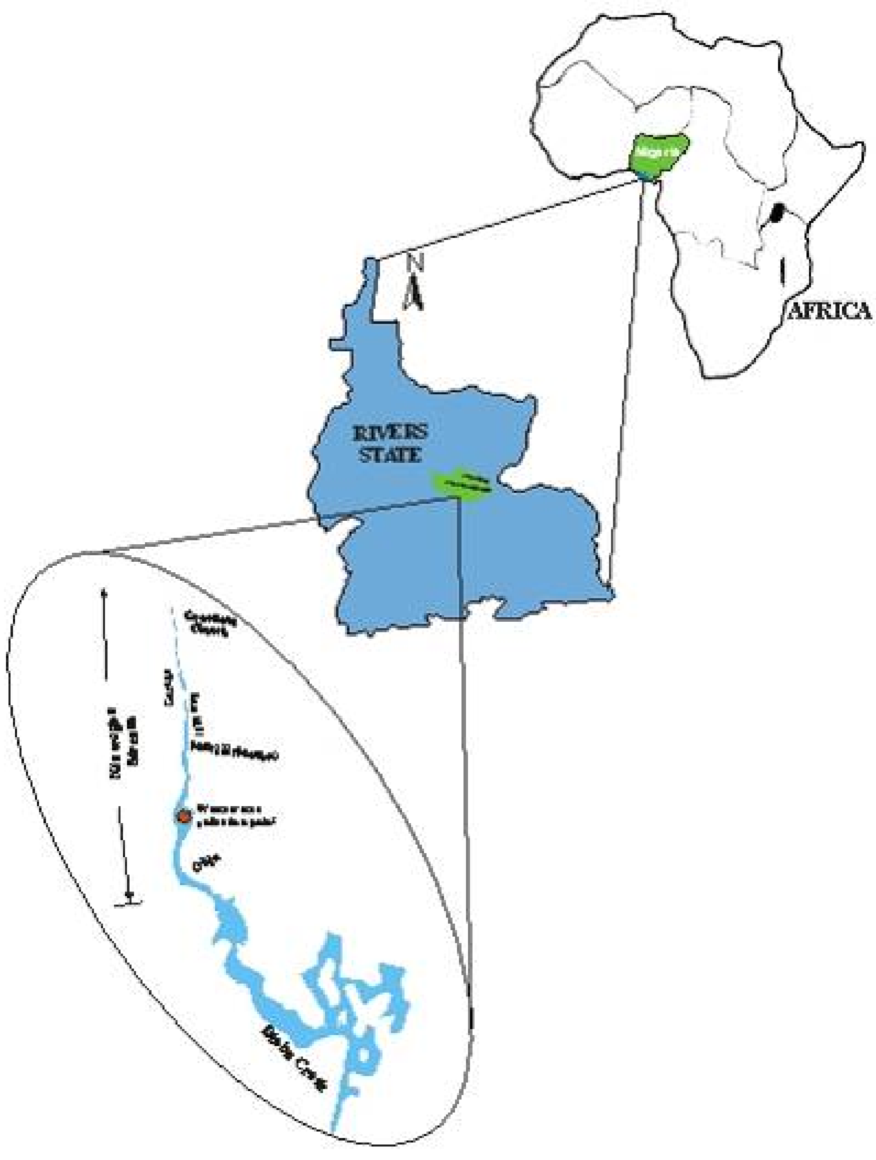

Figure 1. Map of Africa, Nigeria, Rivers State and Port Harcourt showing sampling locations.

Sampling collection and laboratory procedures

Physicochemical Parameters

Samples were collected daily with 2ml plastic containers at sub-surface level and analyzed in the Institute of Pollution Studies (IPS) laboratory using procedures as outlined in Standard methods for the examination of water and wastewater (10). Temperature was measured using a mercury bulb thermometer. pH was measured with a pH meter (Hanna instrument model HI8314). The conductivity was measured using the Horiba water checker model U-10. Dissolved oxygen (DO), and biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) were determined using Winkler's method as described in APHA (1998). Other parameters such as ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), sulphate (SO_4^{-2}), and phosphate (PO_4^{-3}) concentrations were determined spectrophotometrically (Spectronic Spectrophotometer 21D), following the procedures as described in APHA (1998).

Biological Parameters

Periphyton

Periphyton was collected daily for a period of 15 days. For each treatment a total of 3 scrapings were taken by removing a slide from the rack seeded in the wastewater. An area of 1cm^2 from each slide was carefully scraped with a sharp edged scalpel. The first scrapings was emptied into a plastic vial containing 20 ml of Lugol's solution for species identification and numeric analysis; and the second scrapings was put in a glass vial containing 5ml of 90% acetone for *chlorophyll a* analysis (biomass).

From the original stock sample, duplicate samples for numerical analysis were obtained by collecting 1ml sub-sample of the properly homogenized sample with a Stampel pipette. The content transferred into a Sedgewick–Rafter counting chamber for enumeration at a microscope magnification of 400x, and identification at magnification of 1000x using the reports of Mills (1932) Sieminiska (1964) Starmach (1974) Patrick and Reimer (1966) Durand and Leveque (1980) and Chindah and Pudo (1991).

The chlorophyll *a* pigment (as mg chlorophyll cm^{-2}) was determined following Standard methods (APHA 1998). Upon removal from the slides the material was immediately transferred to labeled tubes containing 5ml of acetone, which was added to the sample in the plastic vials. This was centrifuged at 450rpm . The supernatant was carefully transferred to a glass cuvette and absorption measured at 630nm, 645nm and 660nm using spectrophotometer (Spectronic 21D).

$$\text{mg/chlorophyll a/cm}^2 = \frac{\text{Ca x Volume of extract (l)}}{\text{Area of substrate (cm}^2\text{)}}$$

Statistical analysis

Species richness, species diversity index, dominance and evenness were analyzed as indicated below.

The species diversity index was determined using the Shannon-weaver's (1964) function H′ given by the equation:

$$H' = -\Sigma\ (ni/N)\ \mathrm{Log}\ (ni/N)\ i$$

Where:

ni = The number of species in group (i),
N = Total number of species in (i) group.

The specie dominance index was calculated using the Bergen-parker dominance index (Chellappa 1990):

$$d = n_{max}/N_T$$

Where:

n_{max} = number of individuals of the dominant species,
N_T = total number of individuals of all the species recorded.

Physico-chemical and biological parameters were analyzed using 2-way analysis of variance (ANOVA). F-test, was conducted evaluate any significant difference between days. Inter-relationship between physicochemical and biological attributes was evaluated using Excel package 2003. Regression model was used to predict the relationships between the actual and expected values amongst some critical variables (physicochemical and biological parameters) and all calculations were performed for n = 16 observations.

RESULTS

Physicochemical parameters

The synopsis of physico-chemical changes observed during the treatment process is presented in Table 1.

Biological parameters

Species occurrence and successional patterns

A total of 50 taxonomic species occurred in the periphyton. These species were represented by Chlorophyceae (17 species), Cyanophyceae (14 species), Bacillariophyceae (11 species) and Euglenophyceae (8 species) (Table 2 and Figure 2). Generally, the emergence of species in the periphyton community differed from one species to another species (Figure 2).

The species dominant in the early stage of the study (1-2days) were euglenin forms (*Euglena pascheri, E. acus,* and *Phacus acuminatus*) and constituted 77.5% of the periphyton community. Thereafter, the euglenin population quickly declined. The decline observed, euglenin was promptly occupied notably by green algal forms (*Chlamydomonas spp.* and *Chlormonas ulla*) in day 3 and 4, they constituted about 54.5% of the population. The presence of these forms gradually faded and was replaced by the cyanobacteria, which represented 62% to 87.9% of the periphyton standing stock. Amongst the cyanobacteria, the dominant species were *Anacystis aeuroginosa, Oscillatoria terebriformis, O. chlalybaea,* and *Lyngbya pseudospirulina* (Figure 2). The gradual disappearance of the blue green algae gave rise to diatoms on day 14. The dominant diatom species were *Synedra acus, S. parasitica, Navicula minima, N. mutica, Nitzschia linearis, Achnanthes linearis,* and they constituted 83% of the periphyton standing stock (Figure 2).

Species richness fluctuated considerably, maintained almost uniform value for the first 2 days (14 species) before an increase to the day 4 (21 species) subsequently declined on the day 5 (15 species) before another increase and stable value between 6th and the 7th day (22 species). After the 7th day a depression in species number was observed on the 8th day (10 species) and species richness increased steadily to attain the second peak on the 10th day (23 species), declined slightly before attaining the maximum value of 50 species to the end of the experiment (Figure 3a).The species diversity increased initially to the 4th day and fluctuated there after demonstrated similar pattern as described for species richness but the peaks (minimum and maximum) did not occur on the same days (Figure 3b). While species dominance index was stable from day1 to day 9 (0.0022), the value increased sharply on day 10 (0.2825) and fluctuated thereafter to the end of the study (Figure 3b) such that dominance index and species richness demonstrated inverse relationship with the other (Figure 3b).

Changes were observed in the periphyton community structure pattern that demonstrated variability at different stages with first development being the encrusting of Euglenophyceae (72.8-

Table 1. Physicochemical variables in the wastewater treatment system from Diobu in Port Harcourt, Nigeria.

S/no parameter	Range	Mean and SD	% Recovery
Temperature (ºC)	26.5 - 32	29.24 ± 2.16	ND
pH	7.2 - 9.0	7.91 ± 0.50	80.00*
Conductivity (μScm^{-1})	506 - 706	620.87 ± 70.26	72.94
Turbidity (NTU)	3 - 62	22.67 ± 13.36	95.2
TDS (mg L^{-1})	358 - 494	440.2 ± 45.81	27.1
TSS (mg L^{-1})	1.74 - 3.19	2.746 ± 0.52	45.5
DO (mg L^{-1})	0.23 - 6.00	2.01 ± 2.15	96,0
BOD_5 (mg L^{-1})	0.92 - 28.5	16.25 ±11.86	96.8
COD (mg L^{-1})	0.81 - 19.95	11.38 ± 8.31	96.8
Nitrate (mg L^{-1})	0.04 - 0.64	0.22 ± 0.16	93.75
Phosphate (mg L^{-1})	0.39 - 4.54	2.83 ± 1.36	91.4
Sulphate (mg L^{-1})	8.81 - 16.01	12.46 ±2.82	45.9

ND – not determined, * increased value

77.6%), followed by the entrant of Chlorophyceae from day 3 to 4 (44.8-54.5%). Cyanophyceae dominated the periphyton community from day 5 to 11(62-87%), while Bacillariophyceae was observed from day 12 to 15 (57.6-83%), in that respective order (Figure 4). These episodic dominance by major taxonomic groups influenced series of patterns observed, such that at the early (day 1 to 2) stages, encrustation pattern was in the decreasing order of Euglenophyceae (77.6%) > Cyanophyceae (14.2%) > Chlorophyceae (8.2%) Bacillariophyceae (0%). Thereafter the changes in encrustation progressed at the mid stages particularly on the 8th day with a community structure pattern of Cyanophyceae (54.4%) > chlorophyceae (22.6%) > Euglenophyceae (19.5%) > Bacillariophyceae (3.5%). At the end of the study another shift in community structure was observed which followed a sequence of Bacillariophyceae (57.6%) > chlorophyceae (39.4%) > Cyanophyceae (1.7%) > Euglenophyceae (1.2%) respectively (Figure 4).

Periphyton standing stock was observed to maintain the same trend as was observed for species dominance index pattern throughout the study duration. The highest standing crop of (63111 × 10^2 indiv/cm^2) was obtained on day 10, while the least standing crop of (287 × 10^2 indiv/cm^2) was obtained on day 8 of the study (Figure 5a). It was observed that, periphyton standing stock had direct relationship with dominance index (D), but exhibited inverse relationship with species diversity index (H').

Periphyton Biomass (chlorophyll *a*)

Similarly, chlorophyll *a* increased from a minimum of (0.0033μg/cm^2) on day 2 to a remarkable maximum increase (1.6994 mg/cm^2) on day. The chlorophyll *a* concentration also demonstrated a strong affinity with species dominance index and standing stock (Fig. 5b).

Amongst the periphyton descriptors chlorophyll *a* (R^2= 0.66) was the best regressed followed by periphyton densities (R^2= 0.47), Species richness (R^2= 0.24), species diversity (R^2= 0.16) = species dominance index (R^2= 0.16)

Table 2. The periphyton species observed in the treatment tank during the depuration study

Family	Species	
Cyanophyceae	*Anabaena flos-aquae* (Lyng) Breb	*Chroococus minuta* Skuja
	Anabaenopsis arnoldis Aptkarj	*Gloeocapsa magna* (Breb) Kütz
	Anacystis aeuroginosa Kütz.	*Gomphosperia aponina* Kütz.
	Chroococcus turgidus (Kützing) Nägeli	*Lyngbya pseudospirulina* Pascher
	Rhabdoderma lineare Schmidle et Lauterborn	*Merismopedia punctata* Meyen
	Oscillatoria chalybaea (Mertens) Gom.	*Oscillatoria okenii* (Ag.) Gom.
	Oscillatoria terebriformis (Ag.) Gom.	*Romeria elegans* (Wolosz.) Kocz
Chlorophyceae	*Chlamydomonas reinhardtii* P.A. Dangeard	*Ulothrix limnetica* Lemmerman
	Chloromonas ulla (Skuja) Gerloff et Ettl.	*Closterium incurvum* Bréb.
	Euastropsis richteri (Schmidle) Lagerheim.	*Closterium limneticum* Lemm.
	*Scenedesmus acornis (*Ehr.*)*	*Cosmarium pyramidatum* Bréb
	*Scenedesmus quadricauda (*Turpin) Breb.	*Coelastrella levicostata* Korshikov
	Scenedesmus ovalternus (Bernard) Chodat	*Phacotus lendneri* Chodat
	Scenedesmus obliquus (Breb) Playfair	*Tetradesmus crocici* Fott et Kom
	Scenedesmus pseudoarmatus T. Hortobágyi	*Staurastrum apiculatus* (Scott & Prescott) Croasdale & Scott
	Roya cambrica West & G.S.West	
Euglenphyceae	*Phacus granum* Drezepolski	*Euglena acus* Ehr.
	Phacus acuminatus Stokes	*Euglena pascheri* Swirenko
	Phacus pleuronectes (O.F. Müller) Duj.	*Lepocinclis teres* (Schmitz) Francé
	Trachelomonas zuberi Koczwara	*Lepocinclis steinii* Lemm.
Bacillariophyceae	*Achnanthes linearis* (W. Sm.) Grun.	
	Achnanthes exigua Grun.	*Synedra parasitica* (W. Smith) Hustedt
	Pinnularia maior (Kützing) Cleve	*Navicula minima* Grun.
	Synedra ulna (Nitzsch) Ehr.	*Navicula mutica* Kütz.
	Synedra acus Kütz	*Navicula cuspida* Kutz.
	Nitzschia linearis (C.A. Agardh) W. Smith.	*Navicula lanceolate* (Ag.) Kütz.

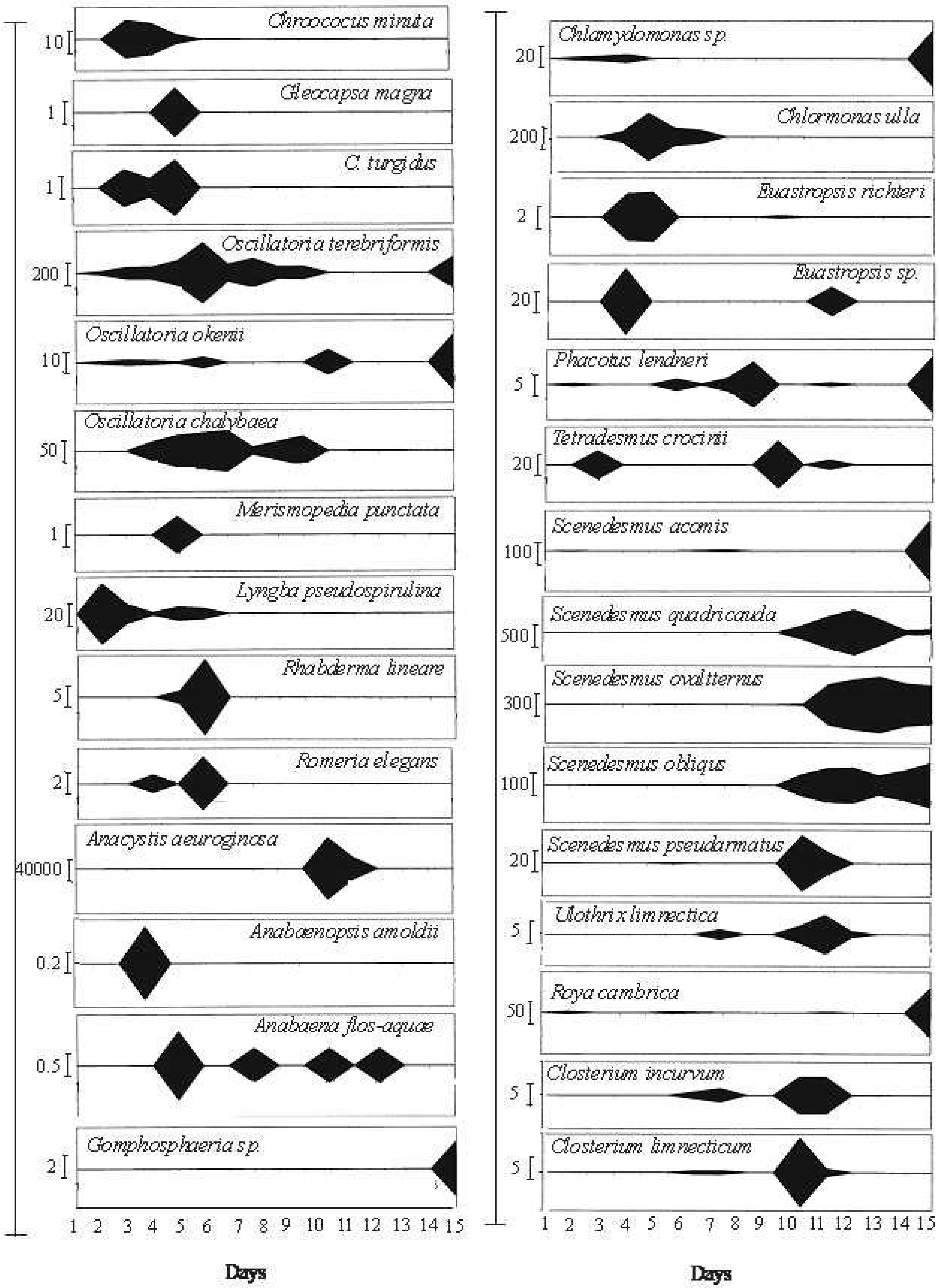

Figure 2. Kite diagram of periphyton species succession in a wastewater retention tank.

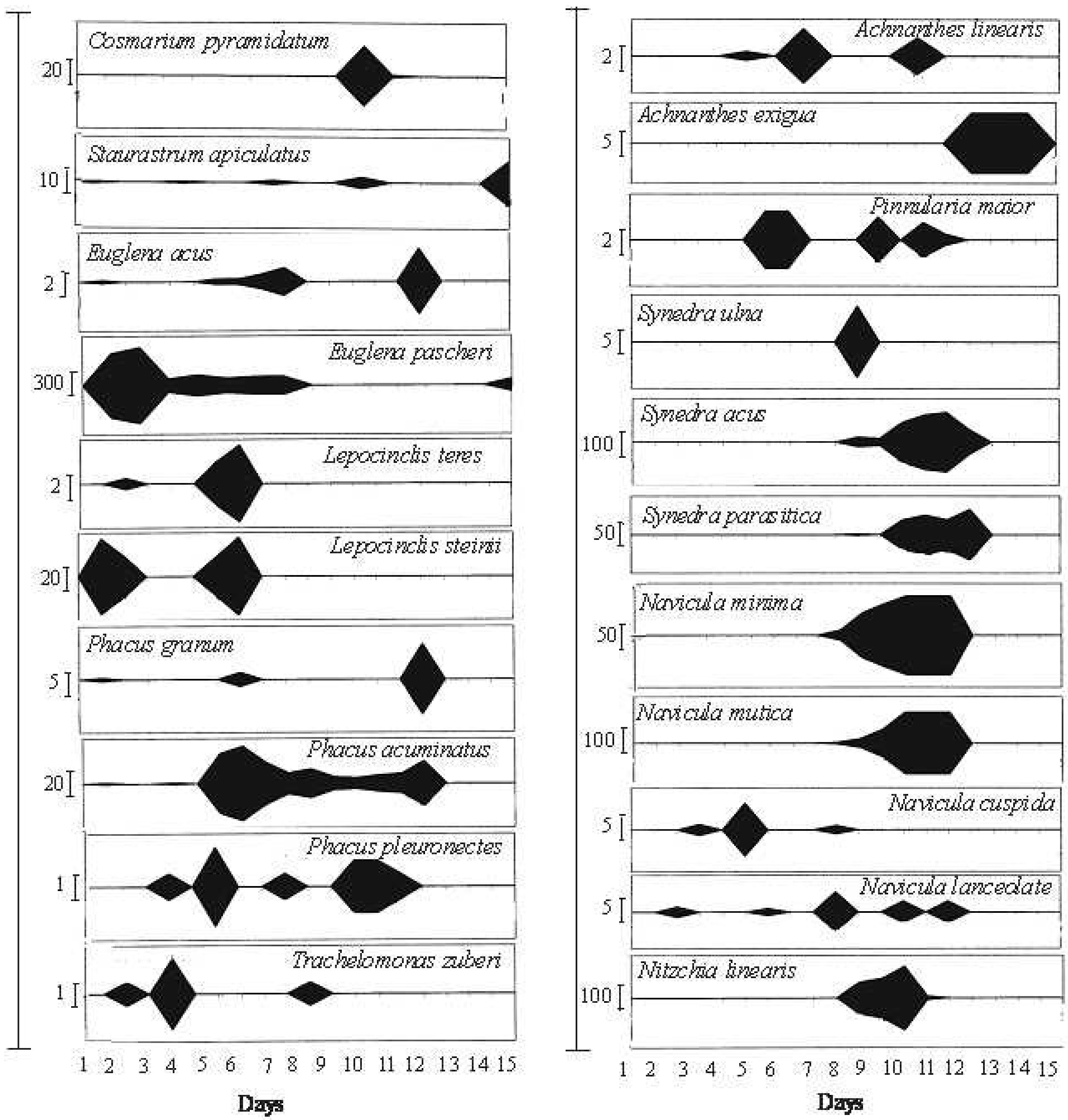

Figure 2. cont. Kite diagram of periphyton species succession in a wastewater retention tank.

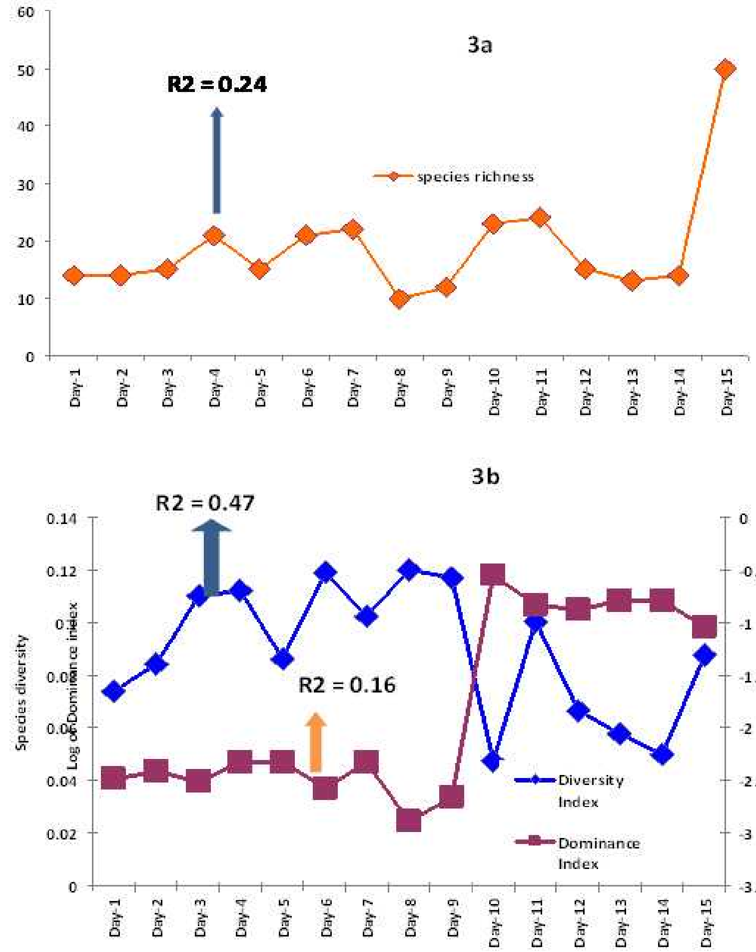

Figure 3. Periphyton species richness (3a), diversity index and species dominant index (3b) in the tanks.

The correlation coefficient recorded some

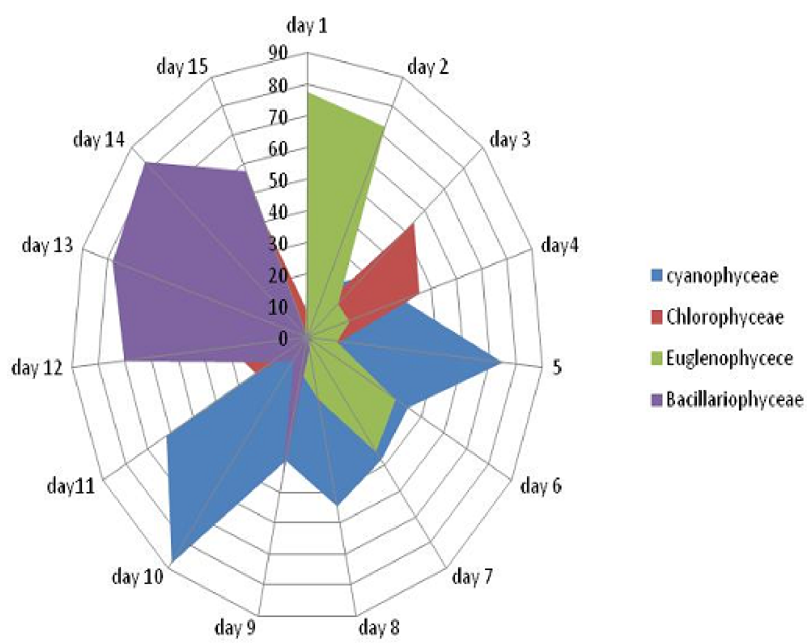

Figure 4. The relative composition of periphyton community at each of the sampling occasion.

relationship between the dependent and independent (physicochemical and biological attribute). Strong positive relationships were observed between exposure period and biomass (chlorophyll *a*), DO, pH, abundance, and dominance index. Chlorophyll *a* exhibited strong positively correlation with DO, pH, abundance and Dominance index. Other strong positive relationships were that between PO_4 with Conductivity and TDS, SO4 with conductivity, turbidity, and TDS and pH with Dominance index. Moderate positive associations were observed between conductivity with turbidity and TDS; BOD_5 with conductivity, turbidity, TDS, and TSS; NO_3 with conductivity, turbidity, and TSS, SO4 with NO_3 and PO_4; pH with DO and abundance; and PO_4 with Species diversity. Low positive relationships were observed between Turbidity and TDS; NO_3 with BOD_5, COD, and PO_4; DO with temperature, abundance and dominance index; and SO_4 with COD. Strong inverse relationship were also observed such as the relationship between exposure period with conductivity, TDS, NO_3, PO_4,and SO_4; chlorophyll "a" with conductivity, TDS,PO_4; pH with Conductivity, TDS, NO_3 and SO_4; Species diversity with abundance and dominance index. Moderate inverse relationships were observed between exposure period with Turbidity, BOD_5, and COD; chlorophyll *a* with turbidity, BOD, NO_3, SO_4 and COD; Conductivity with DO, abundance, and dominance index; DO with conductivity, turbidity, DO, COD and

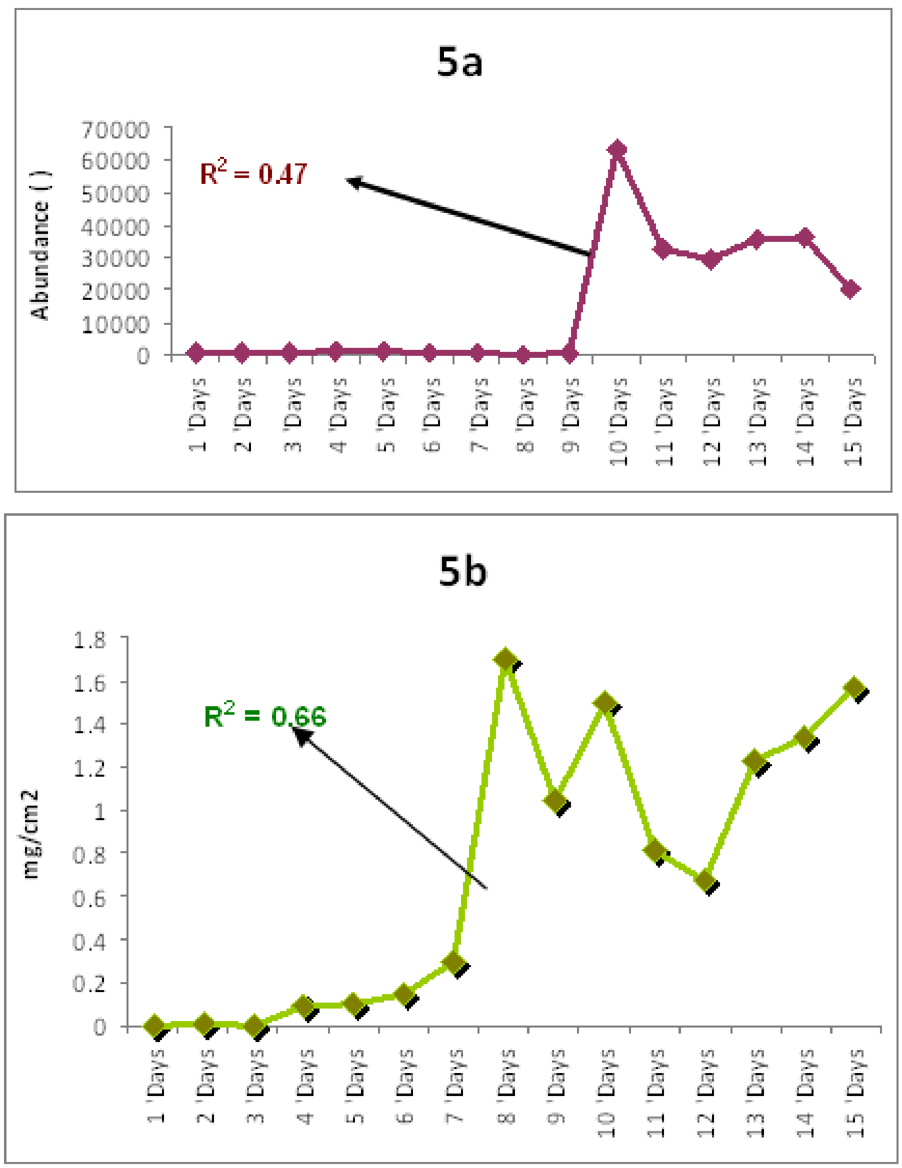

Figure 5. The abundance (a) and chlorophyll *a* concentrations (b) for the various periods

TDS; TDS with DO, abundance and dominance index; TSS with COD; PO4 with pH, abundance, and dominance index; SO_4 with abundance, dominance index; pH with COD and species diversity index. Low negative associations were observed only between species diversity with exposure period and chlorophyll *a*.

The linear regression model

The predictions on the response of different water quality parameters during the exposure time and the relationships with the actual data set from this study were analyzed using the linear regression model presented in Figures 6a-j.

The model showed the relationship between chlorophyll *a* and some measured attributes indicated that while some attributes such as exposure time, DO, dominance index, PO_4, pH, and SO_4 were more strongly correlated to the changes in chlorophyll *a* as they explained 90, 77, 76, 74 72 and 67% of the variation respectively (as expressed in equations 1, 2, 3, 4, 5 and 6).

Equation 1:

$$\text{Chlorophyll } a = 0.1177(t) - 0.3079 \ (r2= 0.90)$$

Where t is duration of exposure, r^2 (90%) being changes in chlorophyll a attributed to exposure period.

Equation 2:

$$\text{Chlorophyll } a = 0.2081DO + 0.2153 \ (r=0.77)$$

Equation 3:

$$\text{Chlorophyll } a = 4.9688SDI + 0.3019 \ (r = 0.76)$$

Equation 4:

$$\text{Chlorophyll } a = -0.316PO4 + 1.5298 \ (r =0.74)$$

Equation 5:

$$\text{Chlorophyll } a = 0.8422_{(pH)} - 6.0308, \ (r = 0.72)$$

Equation 6:

$$\text{Chlorophyll } a = -0.1399SO4 + 2.3769 \ (r = 0.67)$$

While other contributors with relatively weaker contribution to changes in chlorophyll *a* are NO3, COD, BOD, species diversity with contributing influence to in the order of 58, 54 , 53 and 48% respectively (as expressed in equations 7, 8, 9 and 10).

Equation 7:

$$\text{Chlorophyll } a = -2.0475(NO3) + 1.1034 \ (r = 0.58)$$

Equation 8:

$$\text{Chlorophyll } a = -0.038 \ (COD) + 1.0658 \ (0.54)$$

Equation 9:

$$\text{Chlorophyll } a = -0.0266(BOD) + 1.066 \ (r = 0.53)$$

Equation 10:

$$\text{Chlorophyll } a = = -11.095(SDI) + 1.6215 \ (r = 0.48)$$

In addition, chlorophyll *a* as biomass can be predicted from a combination of some critical water quality attributes (DO, BOD and pH) as represented by a linear regression model in Equation 11):

Equation 11:

$$\text{Chlorophyll } a = - 3880 + 0.1553_{Do} + 0.0054_{BOD} + 0.5207_{pH}, \ r^2 = 0.7007, n = 15.$$

This indicated significantly that 70% of the changes in the chlorophyll *a* concentration could be attributed to the values of DO, BOD, and pH in the wastewater.

Similarly, the prediction can also be defined, using nutrient parameters ($N0_3$, $P0_4$, and $S0_4$) and is represented by a linear regression model in Equation 12.

Equation 12:

$$\text{Chlorophyll} - a = 1.9636 - 0.8620_{N03} - 0.0215_{P04} - 0.0419_{S04}, r^2 = 0.6485$$

Thus, 64% of the changes in the Chlorophyll *a* can be attributed to $P0_4$, $S0_4$, and $N0_3$ values on the coefficient of determination r^2.

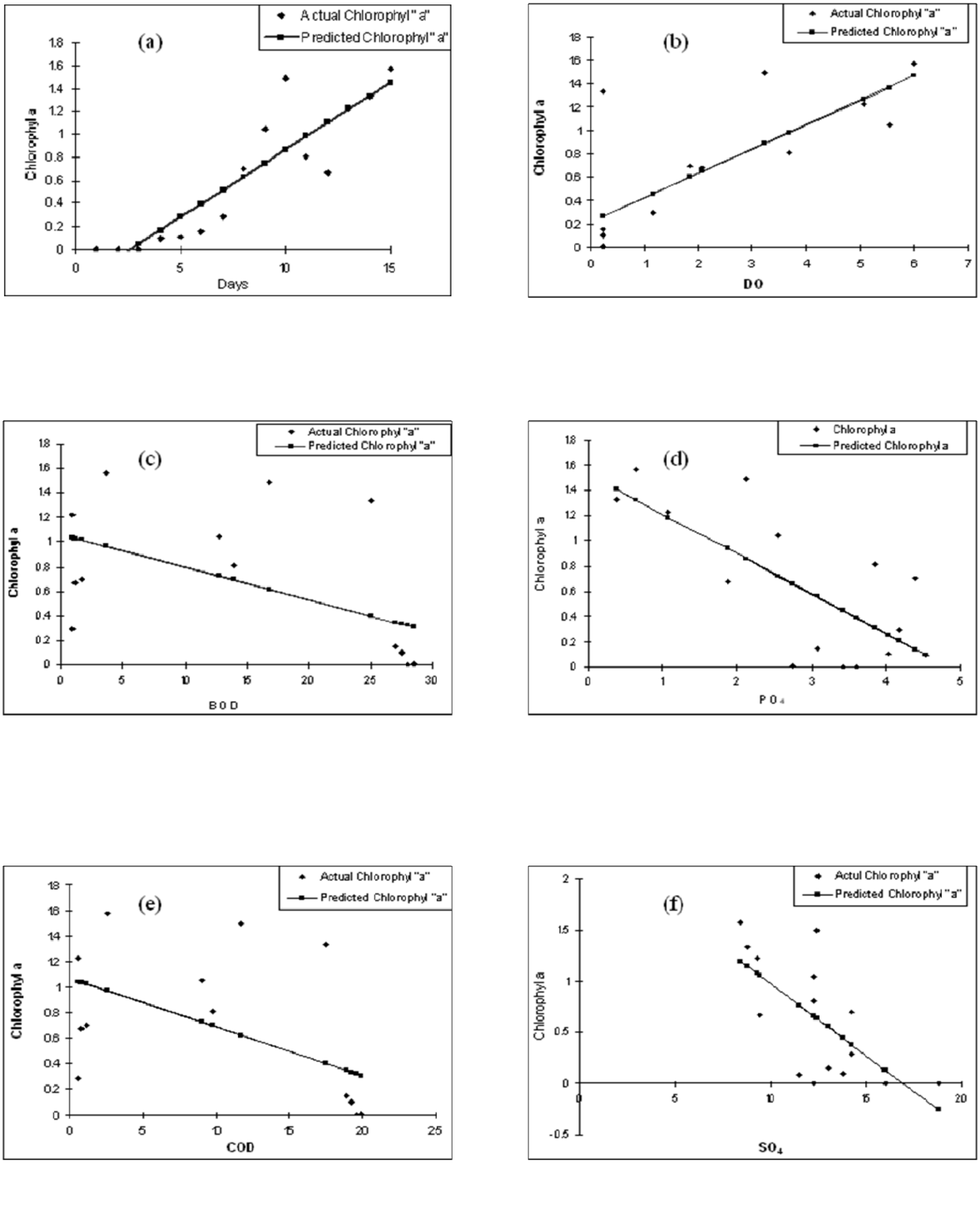

Figure 6. Relationship between chlorophyll *a* and other variables: (a) exposure time; (b) DO; (c) BOD; (d)PO_4; (e) COD and (f) SO_4.

DISCUSSION

The physico-chemical changes observed during the treatment process demonstrated considerable changes for most of the parameters as reported earlier in a separate report (Chindah *et. al*. 2005)

The total number of species encountered in the periphyton community during the study was lower than that observed for the phytoplankton in the same treatment medium (Chindah *et al.,* 2007). The reasons for the differences may be associated with the fact that all the emerging species in medium may not be periphytic in nature. However, most of the species observed in the periphyton community have been reported in natural stream systems in the Niger Delta region (Chindah 1998; Chindah *et al.,* 1999b). The lower number of species richness observed in the treatment medium vis-à-vis that of natural water bodies is expected due to continuous and longer period of exposure and interaction with changes in the water regimes. Nonetheless the phytoplankton pooled higher species richness than the periphyton community that recorded lower species richness. This differences observed in species richness may be associated with the duration as there may not have been adequate retention as is the case in natural water bodies (Chindah 2003: Chindah *et al*., 1999b).

The observed increase over time in periphyton species recruitment and development of periphyton community suggest that such increment in species probably may be alluded to individual species requirement to changes in nutrients and other important environmental gradient factors regulating the pattern observed in the tank. These factors probably are responsible for the observed sequence in the entrant of these species at certain water quality. This is possibly evidence supporting the response and preference of periphyton species to different water quality. Such predilection influence recruitment

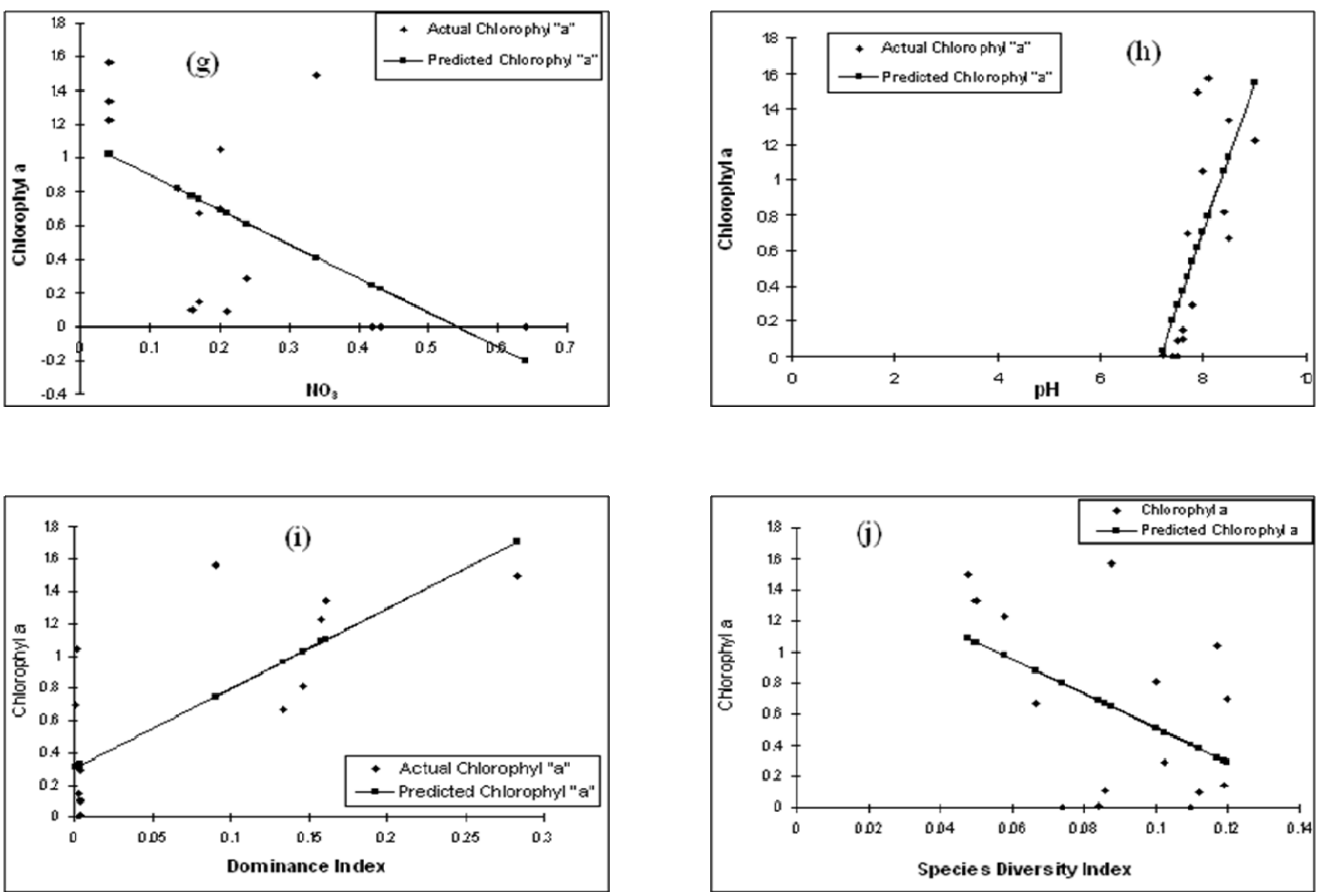

Figure 6. cont. Relationship between chlorophyll *a* and other variables: (g) NO_3; (h) pH; (i) Dominance index and (j) Species diversity index.

pattern as previously reported for phytoplankton community under similar circumstance (Chindah, 2007), but the periphyton community differed in some of the species types and in the recruitment and prosperity pattern. This observation corroborates findings of other scholars that reported distinct changes in periphyton community as the nutrient gradient progresses (Lakos *et al.*, 1997; Pringle 1990; Pan *et al.*, 2000; Hillebrand and Sommer, 2000).

The initial occurrence of euglenins, green and blue green algal species especially the species of *Oscillatoria terebriformis, Lyngbya pseudospirulina, Chlamydomonas reinhardtii. Euglena pascheri, Lepocinclis steinii*, and *Oscillatoria chalybaea* suggest that these are not sensitive species and or species that are resistant and or indifferent to such increases or even favored by such conditions (opportunistic species) as the municipal wastewater stressors. Those species therefore are more tolerant to the stressor where excluded from the population and can be classified as tolerant species. This also qualifies these species as indicator species for waste water monitoring.

However, the later emergence of diatom species that were absent in the early stages suggest that the waste water contains contaminants that negatively suppress the development of these species that were absent at the early stages. This observation is congruent with remarks on other studies in crude oil contaminated environment (Amadi *et al.*, 1997; Chindah 1998; Pudo and Fubara 1998; Chindah *et al.,* 1999b). EPA (2002) contends that municipal and industrial wastes favour the occurrence and preponderance of some algal species over others especially those species that have the ability to tolerate unfavorable and extreme conditions. However the composition of species at the end of the study is similar to trends observed in natural soft acid freshwater system (Chindah 2003). Thus the improved complexity in species composition in the periphyton community provided ample evidence suggesting that the depuration resulted in improved water quality thus responsible for improved status in periphyton species richness and its diversity. This is in consonance with the observation of Eloranta, 1999 who observed that diatoms community reacts with changes in water quality within a few days.

Species richness and diversity were observed to decrease and increase in an oscillating pattern but in relatively similar manner throughout the study. This pattern observed may be associated with the shifts in dominance of the periphyton community. This is in agreement with previous findings in a freshwater stream by Stevenson *et al.,* (1991), Hillebrand and Sommer (2000ab), Stevenson *et al.*, (1991) and who independently observed that decreases in diversity with colonization time was due to an increased dominance of some algal species. However, Falomo (1988), Hilleband *et al.,* (2000) and McCormick (2001) attributed such changes in marine environment to alterations in nutrient levels. The high species dominance index is indicative of the high nutrient concentration and periphyton standing stock in the wastewater. This result and those by Boyton *et al*., 1983; Falomo 1988; Stevenson *et al.,* 1991 and Sabater *et al*., 1998) confirm that proliferation of algal species resulted in high dominance index. Consequently an inverse relationship was observed between species dominance index and species richness and species diversity index,

The shift observed in the community structure from the beginning to the end of the study such that Cyanophyceae >Bacillariophyceae > Euglenophyceae > Chlorophyceae in decreasing order of importance is similar to other studies on the impact of sewage discharges on the water quality and periphyton communities (Pudo 1985; Chindah 1998 and Chindah *et al*., 1999b). The observed reversal in role in the community structure from the early to the middle and to the end of the study suggests on one hand that changes of individual species of different taxonomic groups and abundance over time and on the other hand on competitive ability for nutrient, substrate surface area and light availability. Earlier studies (Jackson 1977; Hoagland *et al*., 1982 and Chindah *et al*., 1999b) reported similar results in their periphyton assemblages.

The dominance of the diatoms species at the later stages of the experiment is indicative of its positive response to increase DO and reduced BOD_5 and nutrient levels, which connotes improved water quality status. Conversely, the early dominance of euglenin and blue green algae species is indicative of its firstly attributed to there preference or tolerance of low pH and DO and high BOD_5 and nutrient suggesting poor water quality. This result is in agreement with previous reports by Amadi *et al.,* (1997), Chindah (1998) and EPA (1990, 2002), that the preponderance of blue algae over other forms is indicative of an altered community structure and poor water quality and the increase of diatoms species is suggestive of a community that had attained stability (Chindah *et al*., 2007).

The periphyton standing stock and biomass were exceptionally higher on day 8 and 10 than those from other days. This may be attributable to the preponderance of blue green algal forms over others during the corresponding period. There have been similar findings by other researchers on primary producers, positing that high proportions of blue green algae contributed significantly to periphyton abundance and biomass (Brock 1985; Pudo *et al.,* 1988; Pudo 1989; Vymazal and Richardson 1995).

However, Soler *et al.,* (1991) in their studies observed that high biomass concentration coincided with the blooms of *chlamydomonas* (green algae) in self-depuration of wastewater body. Form our study; it is difficult to draw such conclusions as maximum chlorophyll *a* was observed when there were reasonable entrants of species from other family groups in the periphyton community. It is therefore possible to suggest that chlorophyll *a* concentration in wastewater treatment system dependent on the blooms of the different species possibly due to the fact that the present study did not consider other chlorophyll types.

The periphyton standing stock and biomass were higher than those reported in natural black water stream in the region, with considerable lower nutrient quality status (Chindah 2003; Amadi *et al*., 1997). It is therefore possible, to associate the differences in periphyton standing stock and biomass to nutrients. This observation is in agreement with earlier reports by Borchardt (1996) that reported that high nutrient availability in a medium yielded high periphyton abundance and biomass.

The inter-relationships of the physiochemical and biological parameters as reflected in the correlation coefficient matrix that gives an overview of the role of the water quality variables on the periphyton community. The strong positive associated observed between exposure period and some biological attributes (Biomass, chlorophyll *a*), abundance, and dominance index and physicochemical variable (DO, pH,) suggested that exposure time played a key role on these attributes. Other similar strong positive association such as the relationship between biomass (chlorophyll *a*) in one hand with DO and pH; and secondly with abundance and dominance index implies that these attributes are important and fundamental characteristics in monitoring periphyton in waste water treatment. The medium and low positive associations explained elsewhere in this study demonstrate the critical role played by each of the variable and this is expected in natural phenomenon. Conversely, the strong negative relationship between the concentrations of some nutrient parameters such as PO_4 and SO4 with species diversity, dominance index and periphyton abundance leads to the conclusion, that periphyton species diversity, dominance index and abundance, are favourable under nutrient limitation (Peterson and Grimm, 1992; Alcoverro *et al*., 2000). This phenomenon is attributed to relevance of the nutrient imbalance in the production of extracellular polymeric substances by the benthic or resuspended diatoms under nutrient limitation as posited by Alcoverro *et al*., 2000. Generally it is pertinent to suggest that while some of the variables constitute a defining factor critical to the depuration process, others appear to be of less environmental consequences to the system. This result agrees with previous studies that periphyton biomass decline with increase in nutrient availability and increase in grazing pressure by epizooic species (McCormick and O′Dell 1996; Pan *et al*., 2000; EPA, 2002).

The critical associations observed between the periphyton and water quality highlight the importance of water quality and environmental gradient on the organization of biological resources and the close relationship between the predicted and actual data implies that these parameters can be relied upon in waste water treatment monitoring as they provide understanding of the possible ecologic effects of anthropogenic activities and ecosystem stability. It is the belief of the authors that the study has provided a framework in which ecological processes can be manipulated to achieve a desired phytoplankton community that identifies successional activities and dynamic factors influencing succession in a restoring singularly applied treatments.

It is therefore possible to suggest that while some of the variables are critical to the depuration process, others appear to be of less environmental consequences to the system. The predictive model allowed us to conclude that calculations based on biomass are good descriptors of the studied system, although other units could be preferentially used in other environments.

CONCLUSION

The changes observed in some of the physiochemical and biological parameters in this study are suggestive of the recovery of a wastewater body and such as the reduction of biological oxygen demand, Chemical oxygen demand, Nitrate and Phosphate concentrations, as well as the increase in species composition of periphyton assemblages that are indicative of a more stable aquatic environment.

The periphyton standing stock and biomass have direct relationship with species dominance, but exhibit inverse relationship with species diversity.

The detection of the pattern of succession of euglenins → green algae → blue green algae → diatoms, is a very useful tool to discern the stages of the depuration and detect possible future changes in the composition of the periphyton community.

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